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31. A host cell according to claim 30, wherein the cell is a mammalian or insect cell.

32. A host cell according to claim 31, wherein the cell is a Chinese hamster ovary (CHO) cell, human embryonic kidney (HEK) 293 cell or an insect Sf9 cell.

33. A host cell according to claim 30, wherein the cell expresses the NPY-Y7 receptor onto the cell's surface.

Lastly, please cancel claims 15-25 with Applicant reserving the right to file divisional applications thereon.

REMARKS

By this amendment, claims 1, 2, 8, 9, 11-14 and 15-25 are canceled, claims 3-7, and 10 are amended, and new claims 26-33 are added to place this application in condition for allowance. Currently claims 3-7, 10, and 26-33 are pending before the Examiner for consideration on their merits.

In summary, Applicant is now claiming:

1) an isolated polynucleotide as defined by claims 3 and 4;

2) an isolated polynucleotide as defined by claims 5 and 6;

3) an isolated polynucleotide as defined in claim 7;

4) a plasmid or expression vector including the polynucleotide of claim 3 (claim 10);

5) a host cell transformed with the polynucleotide of claim 3 (claim 26), and additional limitations to this host cell as found in dependent claims 27-29; and

6) a host cell transformed with the plasmid or expression vector including the polynucleotide of claim 3 and additional limitations to this host cell as found in dependent claims 31-33.

Applicant wishes to clarify that claim 11 which described a host cell transformed with either the polynucleotide of claim 3 or the plasmid or expression vector of claim 10 has been rewritten into new claims 26 and 30. The further limitations on the host cell as recited in claims 12-14 are rewritten as claims 26-29 for the polynucleotide and rewritten as claims 31-33 for the plasmid or expression

vector. Rewriting these claims in this fashion does not deviate from the subject matter previously b fore the Examiner as found in claims 11-14.

Rejection under 35 USC 101 – utility

Addressing the utility rejection, six members of the NPY receptor family had been identified, characterized before the priority date of the present application and shown to influence a number of physiological parameters such as central endocrine secretion, anxiety and appetite (see, e.g., page 1 lines 12 to 31 of the specification) and to couple to the adenylate cyclase second messenger system.

Given the characterization of the other members of the NPY receptor family already achieved by the priority date of the present application, the identification of a novel family member would present the person skilled in the art with immediate and obvious utility. It is not necessary for the applicant to have established the precise function of the NPY-Y7 receptor disclosed in the present application to fulfill the utility requirement.

Nonetheless, the applicant has, in the present application, conducted Northern blotting studies to show that the NPY-Y7 receptor polypeptide disclosed in the present application is expressed in amygdala, the CA3 region of the hippocampus and piriform cortex, consistent with a neural receptor (see, e.g., page 6 lines 4 to 10 of the specification). The conclusion drawn at page 6 lines 9 to 11 of the specification is that the receptor is involved in the regulation of Circadian rhythms, anxiety and metabolic status.

Applicant has also carried out ligand binding studies and shown that the NPY-Y7 receptor of the present invention shows high affinity for the human peptide YY (PYY) (see page 6 lines 12 to 20). The conclusion drawn is that NPY-Y7 receptor is pharmacologically most like the NPY-Y2 receptor. In addition, confirmation that the NPY-Y7 receptor of the present invention is targeted to the cell membrane where it binds PYY was obtained using PYY binding studies comparing cell membrane preparations from control cells with cell membranes from cells that express recombinant NPY-Y7 of the present invention (page 7 lines 9 to 30)

Human peptide YY had been shown before the priority date of the present application to be involved in the regulation of Circadian rhythms. In support of this, applicant submits a copy of Gribkoff et al., 1998, J. Neuroscience, vol. 18(8), pp3014-3022 (see, especially, the abstract, page 3018, the second paragraph, and

Figure 7). As discussed above, the specification implicates specifically the NPY-Y7 receptor in the regulation of Circadian rhythms.

The data xemplified in the present specification represent a significant biochemical characterization. Furthermore, the finding that NPY-Y7 binds PYY with high affinity is significant because PYY itself has been extensively characterized in the prior art. See the attached three website printouts of abstracts related to PYY. Consequently, since PYY has been extensively characterized in the prior art and NPY-Y7 binds PYY with high affinity, a substantial and credible utility for NPY-Y7 is established by these experimental data.

Thus, Applicant respectfully disagrees with the Examiner's assessment that the only asserted utilities are non-specific and "solely based on the fact that NPY-Y7 is a member of the NPY family of receptors". The specification asserts on the basis of the actual experimental result presented therein in relation to the neural distribution of mRNA expression and pharmacological characteristics, that the receptor is involved in the regulation of specific physiological parameters, namely the circadian rhythm, anxiety and metabolic status.

Accordingly, whilst the specification does, as noted by the Examiner, assert that the NPY-Y7 receptors of the invention would be useful both clinically and commercially, for example in identifying agonists and antagonists of the receptor (see, e.g., page 1 line 32 to page 2 line 2; page 3 lines 10 to 13 of the specification) due to the known role of NPY receptors in a number of physiological processes, this general assertion should be read in the light of the more specific utilities that are provided therein.

Thus, in summary, a substantial and specific utility is asserted in the specification, and the rejection under 101 is unsubstantiated and should be withdrawn.

In terms of whether the asserted utility is credible, with respect, where utility is asserted in a specification, the test is whether the person of ordinary skill in the art would consider that the assertion was credible (MPEP 8th Ed. 2107 II.(B)(1)(ii)).

Applicant submits that the assessment made and conclusions drawn in the present specification from the available data and prior knowledge of the biological characteristics of the NPY family would be considered reasonable and realistic by a person of ordinary skill in the art. No substantial evid nce has been provided by the Examiner to conclude otherwise.

Applicant further submits that specification discloses a specific asserted utility, which would be considered by a skilled person to be credible given the knowledge in the art in relation to the NPY family of proteins and their ligands and the specific molecular characterization provided in the specification.

Applicant submits that the initial burden resides with the Office to establish a *prima facie* case for a finding of lack of utility and provide sufficient evidentiary basis for that finding. Applicant respectfully submits that the Examiner has not done so. *In re Gaubert*, 524 F.2d 1222, 1224, 187 USPQ 664, 666 (CCPA 1975) – "the PTO must do more than merely question operability – it must set forth factual reasons that would lead one skilled in the art to question the objection truth of the statement of operability". The Patent Office must establish that it is more likely than not that a person skilled in the art would not consider credible any specific and substantial utility asserted by the applicant for the claimed invention (MPEP 8th Ed. 2107 III.(A)).

It is also noted that the Examiner refers to Example 4 of the USPTO utility guidelines. However, Example 4 refers to a situation where there is no disclosed utility and no description of the chemical, physical, or biological properties for the protein other than the sequence. We respectfully submit that Example 4 of the utility guidelines is not similar to the present circumstance because the instant application has provided a substantial characterization of the NPY-Y7 receptor protein of the present invention and asserted a utility in the specification based on that characterization.

Again, a substantial, specific and credible utility has been asserted in the specification based on actual experimental data and sound scientific reasoning. The Examiner is respectfully requested to withdraw the allegation under 35 USC 101 in view of the foregoing Remarks.

Rejection under 35 USC 112, first and second paragraphs

Applicant asserts that the comments above with respect to utility are equally applicable to the rejections under 35 U.S.C. § 112, first and second paragraph, and form a basis to have these rejections withdrawn.

More particularly, since the specification does meet the requirements of 35 USC 101, one skilled in the art would know how to use the rejection. Therefore, the rejection based on a lack of support that begins on the bottom of page 5 of the Office Action should be withdrawn.

Applicant also wishes to bring to the Examiner's attention that claim 3 is now limited to a length of 408 amino acids. Claim 5 defines the length as 405 amino acids. "About " has been deleted from ach of these claims, and the rej ction of claims 3 and 5 based on written description is effectively overcome.

The objectionable language found in claim 7 has been deleted and claim 7 now recites specific nucleotide sequences.

The cancellation of claims 8 and 9 moots the rejection of these claims.

Based on the amendments to the claims, and the arguments regarding utility above, the claims 3-7, 10, and 26-33 meet the statutory requirements of 35 U.S.C. § 112, first and second paragraphs.

Lastly, since claims 3 and 5 meet the statutory requirements of 35 U.S.C. § 101 and 35 U.S.C. § 112, first and second paragraphs, Applicant respectfully submit that the restriction requirement related to the species of claim 4 and 6 should be withdrawn, and these claims should be passed onto issuance with claims 3 and 5

Applicant also acknowledges that no prior art was cited against the claims.

Summary

Based on the above, claims 3-7, 10, and 26-33 are now in condition for allowance. All statutory requirements under 35 U.S.C. § 112, first and second paragraphs, and 35 U.S.C. § 101 are met.

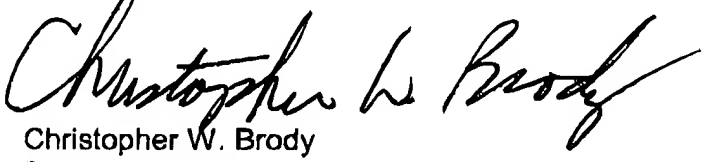
Accordingly, the Examiner is respectfully requested to examine this application in light of this amendment and pass claims 3-7, 10, and 26-33 onto issuance.

If the Examiner believes that an interview would expedite the prosecution of this application, the Examiner is requested to telephone the undersigned at 202-835-1753.

As noted above, a petition for a three month extension of time is hereby made. Please change the petition fee of $920.00 to Deposit Account No. 50-1088. Please charge any fee deficiency or credit any overpayment to Deposit Account No. 50-1088, including extension of time fees.

Again, reconsideration and allowance of this application is respectfully solicited.

Respectfully submitted,
CLARK & BRODY



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Docket No.: 12020-0002
Date: December 19, 2002

Application No. 09/719,088

MARKED UP CLAIMS UNDER 37 CFR 1.121

3. (once amended) An isolated polynucleotide molecule [according to claim 1, wherein the polynucleotide molecule] encod[es]ing an NPY-Y7 receptor of human origin of [about] 408 amino acids in length.

4. (once amended) An isolated polynucleotide molecule according to claim 3, wherein the polynucleotide molecule encodes a human NPY-Y7 receptor having an ~~amino acid sequence [substantially] corresponding to that shown as SEQ ID NO: 2.~~

5. (once amended) An isolated polynucleotide molecule [according to claim 1, wherein the polynucleotide molecule] encod[es]ing an NPY-Y7 receptor of murine origin of [about] 405 amino acids in length.

6. (once amended) An isolated polynucleotide molecule according to claim 5, wherein the polynucleotide molecule encodes a murine NPY-Y7 receptor having an amino acid sequence [substantially] corresponding to that shown as SEQ ID NO: 3.

7. (twice amended) An isolated polynucleotide molecule encoding an NPY-Y7 receptor, wherein the polynucleotide molecule comprises a nucleotide sequence [showing at least 90% homology to that shown at nucleotides 1 to 1903 or nucleotides 369 to 1592 of SEQ ID NO: 4] selected from the group consisting of:

(i) the sequence set forth in SEQ ID NO: 4;

(ii) a sequence consisting of nucleotides 369 to 1592 of SEQ ID NO: 4; and

(iii) a sequence that encodes the amino acid sequence set forth in SEQ ID NO: 2.

10. (once amended) A plasmid or expression vector including a polynucleotide molecule according to claim 3 [1].

The Journal of Neuroscience, April 15, 1998, 18(8):3014–3022

Phase Shifting of Circadian Rhythms and Depression of Neuronal Activity in the Rat Suprachiasmatic Nucleus by Neuropeptide Y: Mediation by Different Receptor Subtypes

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Neuropeptide Y (NPY) has been implicated in the phase shifting of circadian rhythms in the hypothalamic suprachiasmatic nucleus (SCN). Using long-term, multiple-neuron recordings, we examined the direct effects and phase-shifting properties of NPY application in rat SCN slices *in vitro* ($n = 453$). Application of NPY and peptide YY to SCN slices at circadian time (CT) 7.5–8.5 produced concentration-dependent, reversible inhibition of cell firing and a subsequent significant phase advance. Several lines of evidence indicated that these two effects of NPY were mediated by different receptors. NPY-induced inhibition and phase shifting had different concentration–response relationships and very different phase–response relationships. NPY-induced phase advances, but not inhibition, were blocked by the $GABA_A$ antagonist bicuculline, suggesting that NPY-mediated modulation of GABA may be an underlying mechanism whereby NPY phase shifts the circadian clock. Application of the Y2 receptor agonists NPY 13–36 and [Cys[2],8-aminooctanoic acid[3,4],D-Cys[27]]-NPY advanced the peak of the circadian rhythm but did not inhibit cell firing. The Y1 and Y5 agonist [Leu[31],Pro[34]]-NPY evoked a substantial inhibition of discharge but did not generate a phase shift. NPY-induced inhibition was not blocked by the specific Y1 antagonist BIBP-3226; the antagonist also had no effect on the timing of the peak of the circadian rhythm. Application of the Y5 agonist [D-Trp[32]]-NPY produced only direct neuronal inhibition. These are the first data to indicate that at least two functional populations of NPY receptors exist in the SCN, distinguishable on the basis of pharmacology, each mediating a different physiological response to NPY application.

Key words: circadian rhythm; suprachiasmatic nucleus; neuropeptide Y; multiple-unit recordings; phase shifting; receptors

The suprachiasmatic nuclei (SCNs) of the mammalian hypothalamus have been implicated in the control of behavioral and homeostatic circadian rhythms (Rusak and Zucker, 1979; Meijer and Rietveld, 1989; van den Pol and Dudek, 1993). The nuclei receive varied input, including a projection from the retina (Moore and Lenn, 1972; Sawaki, 1979; Shibata et al., 1984). SCN neurons discharge with a circadian pattern, maintained in SCN neurons even in primary culture (Welsh et al., 1995; Liu et al., 1997b). In slices the phase of the rhythm is maintained after slice preparation; the peak firing rate is observed near circadian time (CT) 6 of the light of a 12 hr light/dark cycle in the rat (Green and Gillette, 1982; Gillette, 1991; Bouskila and Dudek, 1993). (Circadian time is a 24 hr scale used to relate experimental measurements to the imposed 12 hr light/dark schedule, with CT 0 representing lights on and the start of the subjective day.) The greatest influence on the rhythm is the light/dark cycle, but it can be influenced by neurotransmitters and neuromodulators including GABA (van den Pol and Tsujimoto, 1985; Tominaga et al., 1994), glutamate (Meijer et al., 1988; Ding et al., 1994), melatonin (McArthur et al., 1991; Liu et al., 1997a), and serotonin (Medanic and Gillette, 1992; Prosser et al., 1992, 1993).

Neuropeptide Y (NPY), a widely distributed neuropeptide (Allen et al., 1983), is present in the SCN (Pelletier, 1990; Botchkina and Morin, 1995), the result of a projection from the intergeniculate leaflet of the thalamus (Moore et al., 1984; Harrington et al., 1985). NPY can produce a phase advance in the circadian rhythm of the SCN (Medanic and Gillette, 1993; Shibata and Moore, 1993; Golombek et al., 1996) and can shift behavioral rhythms (Biello et al., 1994; Huhman and Albers, 1994; Huhman et al., 1996), an effect blocked by the $GABA_A$ antagonist bicuculline (Huhman et al., 1995). NPY also excites or inhibits SCN cells (Mason et al., 1987; Shibata and Moore, 1988; Liou and Albers, 1991; Schmahl and Böhmer, 1997).

At least two NPY receptor subtypes are found in the SCN, Y1 and Y2 receptors (Chen and van den Pol, 1996; Golombek et al., 1996). A mammalian Y5 receptor has recently been cloned and is also found in the hypothalamus (Gerald et al., 1996). In developing SCN, both Y1 and Y2 receptor-mediated inhibition of calcium fluxes produced by activation of bicuculline-sensitive $GABA_A$ receptors can be detected (Obrietan and van den Pol, 1996), and multiple NPY receptors may mediate inhibition of neurotransmitter release (Chen and van den Pol, 1996; van den Pol et al., 1996). Y2 receptor agonists also produce shifts in circadian rhythms in the SCN and produce shifts in behavioral rhythms (Golombek et al., 1996; Huhman et al., 1996). Less is known, however, about the direct effects of NPY on cell discharge in the nucleus or the receptors that mediate these effects and whether direct effects of NPY on cell firing underlie modulation of phase timing.

We used a continuous recording method (Bouskila and Dudek,

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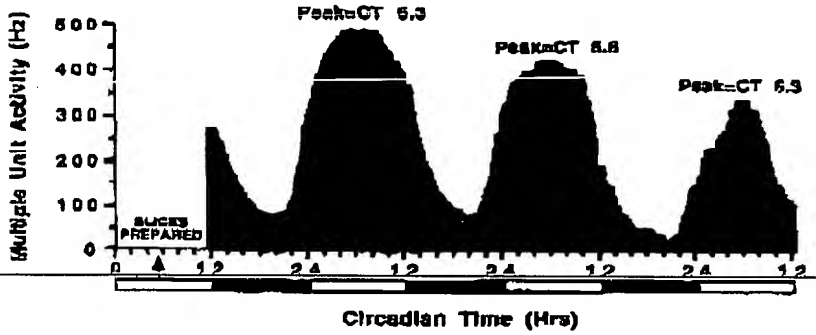

Figure 1. Multiple-unit recording from the rat SCN *in vitro*. In this example the slice was prepared at approximately CT 5.5 on day 1; recording commenced near CT 11.5 on day 1 and continued for nearly three full circadian cycles, ending near CT 12 on day 4. Although the absolute peak discharge rates declined each day, the times at which the discharge rates were maximal were very similar. Values given are estimated peak maximums; peaks were fit and maximums were estimated as described in Materials and Methods.

1993; Tcheng and Gillette, 1996) to record action potentials in SCN slices *in vitro* to study effects of pharmacological manipulation of NPY receptors on SCN function. We used NPY and a series of NPY agonists with differing affinities for Y1, Y2, and Y5 receptors (Gerald et al., 1995), the Y1 antagonist (*R*)-N^2-(diphenylacetyl) - *N* - [(4 - hydroxyphenyl)methyl] - argininamide (BIBP-3226) (Doods et al., 1995), and the $GABA_A$ receptor antagonist bicuculline to examine their ability to affect firing rates of SCN neuron populations and alter the circadian discharge rhythm observed after drug application.

MATERIALS AND METHODS

SCN slice preparation. Male Long–Evans hooded rats (Harlan Sprague Dawley, Indianapolis, IN) were housed in a colony room with an ambient 12 hr light/dark cycle (lights on at 7:00 A.M., lights off at 7:00 P.M.) for a minimum of 3 weeks before experimentation to ensure that their circadian systems were entrained to this light/dark cycle. The rats were housed in stainless steel wire cages, five animals per cage, with food and water available *ad libitum*. After this adaptation period, the rats were killed by decapitation between 9:00 and 11:30 A.M. (CTs 2.0 and 4.5), with one exception that was prepared at CT 5.5 (see Fig. 1), and their brains were rapidly dissected from their skulls. A block of tissue containing the hypothalamus was dissected from the brain under visual inspection and transferred to a manual tissue chopper where coronal hypothalamic brain slices (500 μm in thickness) containing the SCN were prepared. Slices were placed in a Haas-type brain slice chamber (Huner et al., 1979) (Medical Systems Corp.) and continually superfused with medium containing (in mM): NaCl 116.3, KCl 5.4, NaH_2PO_4 1.0, $NaHCO_3$ 26.2, $CaCl_2$ 1.8, $MgSO_4$ 0.8, and dextrose 24.6 and 5 mg/l gentamycin sulfate, warmed to 37°C, pH 7.5. SCN neurons in these brain slices remained viable for >72 hr under these conditions (Medanic and Gillette, 1992), although most experiments were terminated at the end of the second day after slice preparation.

To record multiple-unit SCN electrical activity, a 76-μm-diameter, Teflon-coated platinum–iridium wire electrode (wire diameter, 52 μm) was lowered into the brain slice in the region of the SCN using stable MM33 mechanical micromanipulators (Stoelting, Inc.) (Bouskila and Dudek, 1993) mounted on an air flotation table to eliminate any room vibrations. Physical stability of both the slice and the recording electrode was of paramount importance to obtain reliable recordings. The electrical activity was amplified, and the number of electrical events was counted with a window discriminator (Fintronics, Inc. or Cambridge Electronic Design). Data were collected and analyzed by computer using Brainwave (Data Wave Technologies) or Spike2 (Cambridge Electronic Design) software. The average number of electrical events in successive 10 min intervals was determined and plotted against the circadian time of recording. It was found that portions of the resulting discharge rhythms, which generally corresponded to the upper portions of the phase during the subjective day, were best fit with a cosine function of the form $y = a_1 + a_2x + (a_3x + a_4)\cos(a_5x + a_6)$, where y is the average multiple-unit firing rate in a 10 min period, in hertz, x is the circadian time of recording, and a_1–a_6 are coefficients determined by the Levenberg–Marquardt nonlinear curve fit algorithm (KaleidaGraph, Synergy Software). Curve fitting allowed for smoothing of the inherently variable records from individual slices to more accurately estimate the peak of cell discharge during the subjective day. The peak of this cosine curve was used as a marker of the phase of the rhythm and was used to determine whether the SCN electrical activity rhythm was phase-shifted with drug treatment. An example of this curve-fitting paradigm applied to group data is presented (see Figure 3). Curve fitting could not be adequately performed on a small percentage of slices because of recording irregularities occurring during some portion of the peak on day 2. In these cases, if all other viability criteria were met (see below), data concerning direct effects of drug applications in these slices were included in analyses, although data concerning timing of peaks was not. Conversely, early in the study a small number of experiments were performed in most groups in which the initial period corresponding to the period of direct drug application was not recorded, although the presence or absence of drug effect was noted. In these cases only the effects on peak timing were quantified and presented in the results.

Viability of the slices was considered the most important single factor in reducing variability of control values for day 2 electrical activity peaks, and slices were excluded if their viability was compromised at any point on this second day (a falsely advanced peak could result from a pronounced decrease in slice viability during day 2). Data were accepted from particular slices if (1) the peak activity rates on day 2 were within 30% of the maximal values attained on day 1 (the day of slice preparation), and (2) activity persisted in the subjective night between days 2 and 3 such that the lowest levels of activity were at least 50% of the low values recorded during the subjective night between days 1 and 2. Viability on day 2 was assured if a third peak was observed on day 3; although not all experiments were continued to a point at which the activity peak on day 3 could be determined, most were carried beyond CT 24 (into day 3), and the beginning of a third peak could be observed. Slices were also excluded if an experimental interruption occurred (such as a disruption in medium superfusion) that could affect or obscure recordings made during any critical period or otherwise affect the subsequent shape and timing of the electrical activity rhythm. As a result of the application of these exclusion criteria, ~75% of slices prepared were used in these experimental analyses. Data are presented in most cases for both inhibition and phase shifting as percent change from vehicle or an absolute time shift relative to vehicle. Where appropriate, separate vehicle groups were used for each arm of an experiment. Statistical analyses consisted primarily of ANOVAs; if a significant main effect was detected, preplanned *post hoc* comparisons were performed [Fisher's least significant difference (LSD)], generally to determine which experimental groups significantly differed from vehicle. Statistical treatments are presented in the figure captions for clarity.

Peptides and drugs. NPY (human), (D-Trp^{32})-neuropeptide Y (D-Trp-NPY; human, rat), peptide YY (PYY; human), and (Cys^{5},8-aminooctanoic acid^{6,34},D-Cys^{37}-neuropeptide Y (C2-NPY; human) were purchased from Bachem (Torrence, CA) and/or Peninsula Laboratories (Belmont, CA). [Leu^{31},Pro^{34}]-neuropeptide Y (Leu-Pro-NPY; human), neuropeptide Y fragment 13–36 (NPY 13–36; porcine), and bicuculline (BIC) were purchased from Sigma (St. Louis, MO). BIBP-3226 was synthesized in-house, and its affinity for Y1 receptors was independently confirmed (purity, >95%; K_i for Y1, ~1.0 nM; Y2, Y4, and Y5, >1000 nM, full antagonist in CHO cells expressing human Y1 clonal receptors in an NPY-mediated cAMP stimulation assay; apparent K_b, 4.9 nM) (I. Ansell, personal communication). All peptides were solubilized in deionized water and then diluted with artificial CSF (ACSF) to obtain the desired concentration. Vehicle was 0.4% deionized water in ACSF. All peptides were applied from 2:30 to 3:30 P.M. (CT 7.5–8.5) except in phase–response experiments. In experiments involving coadministration of

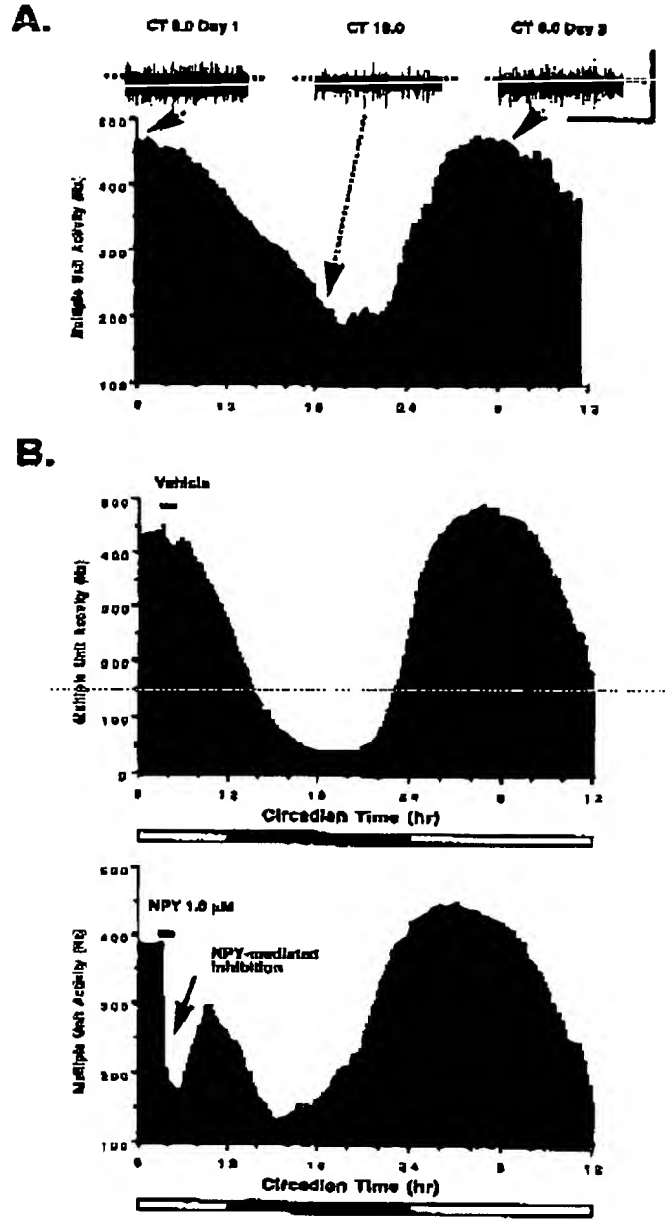

Figure 2. Examples of rhythms generated by 30 hr multiunit recordings from control (*A*) and vehicle- or NPY-treated (*B*) SCN slices. The examples of raw unit recordings in *A* demonstrate the change in discharge frequency between the rhythm maximum (*CT 6*) and minimum (*CT 18*); *dashed lines* are the window discriminator levels. Calibration bars, 1 sec and 200 μV. Note that in the control slice in *A* the peak firing rate on the second day is near CT 6, with the preceding lowest firing level obtained near CT 20. *B*, In other SCN slices, vehicle (*top trace*; see Materials and Methods for vehicle description) or NPY (1.0 μM) was applied between CT 7.5 and 8.5. There was no difference in timing of the rhythm in the vehicle slice. Application of NPY, but not vehicle, resulted in significant direct inhibition of cell discharge, seen as a significant dip in the firing rate, which recovered slowly after a wash into control medium. After the NPY application, the lowest firing rate was observed near CT 15, and the peak discharge rate on day 2 was observed between CT 2 and 3. *Light* and *dark bars* at the *bottom* in *B* represent the light/dark cycle under which the animals were maintained before slice preparation and apply to the trace depicted in *A*.

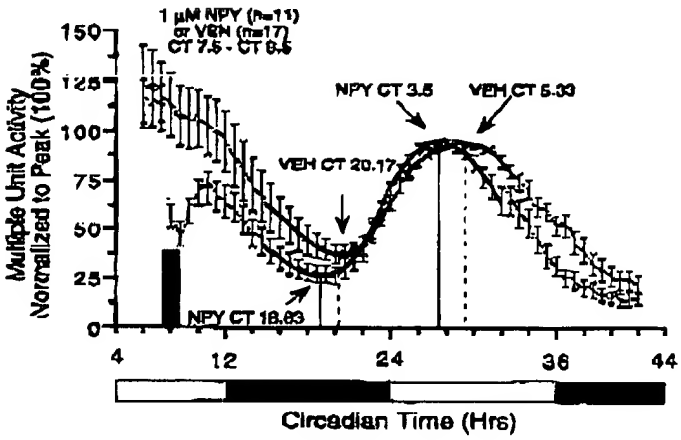

Figure 3. Group data depicting the effects of vehicle and NPY on SCN slices. Groups included slices exposed to 1.0 μM NPY or vehicle and recorded during the duration of the experiment, including NPY administration; a group of slices exposed to 1.0 μM NPY to examine phase shifting only and not included in the grouping for this figure was not recorded during the NPY administration. Values are mean ± SEM. The numerical values given are for the group minimums (subjective night) and maximums (subjective day) for each group, as derived from cosine function fits of the indicated areas of each curve (fitted areas indicated as *darker lines*). Line fitting was for data smoothing near the maximums and minimums only for estimation of these values and was not an attempt to fit entire curves. Although we have not considered this further in this study, note that the mean minimal value is also phase-advanced by NPY, and the mean normalized activity values are depressed during the subjective night, relative to vehicle controls, suggesting very long-lasting effects of NPY treatment.

putative antagonists and agonists, the antagonist was administered alone for 0.5–1 hr before the antagonist and agonist solution, depending on the antagonist (see Results).

RESULTS

Using the multiunit electrode technique, we were able to continuously record cellular activity simultaneously from as many as 15 SCN slices for >2 d with an overall success rate of ~75%. These results include data from 453 SCN slices from an identical number of rats. The remainder of slices, not included in this tally, did not exhibit a normal circadian rhythm on the second day (as defined above). In untreated control slices, circadian rhythms in discharge rate were similar to those reported earlier with this and other techniques. The peak of the firing rate in a group of untreated control slices occurred at circadian time 6.2 ± 0.3 (n = 20, peaks recorded on day 2); peak firing rates on days 1 and 2 were typically two to six times the discharge rate at the low point of the rhythm. Representative examples of recordings obtained from control slices are presented in Figures 1 and 2*A*, including examples of multiunit discharge records in Figure 2*A*. Multiunit records reflected the activity of many cells, and the window discriminator levels were set, in all cases, significantly above the visible noise level, determined before advancing the electrode into the tissue. Whereas few slices were maintained for the ~84 hr depicted in Figure 1, this example demonstrates that recordings could be obtained from SCN slices under our experimental conditions for at least this long. Electrical activity rhythms recorded from this slice, while declining in amplitude each day, maintained peak activity times that were very similar from day to day. In the experiments presented below, experimental com-

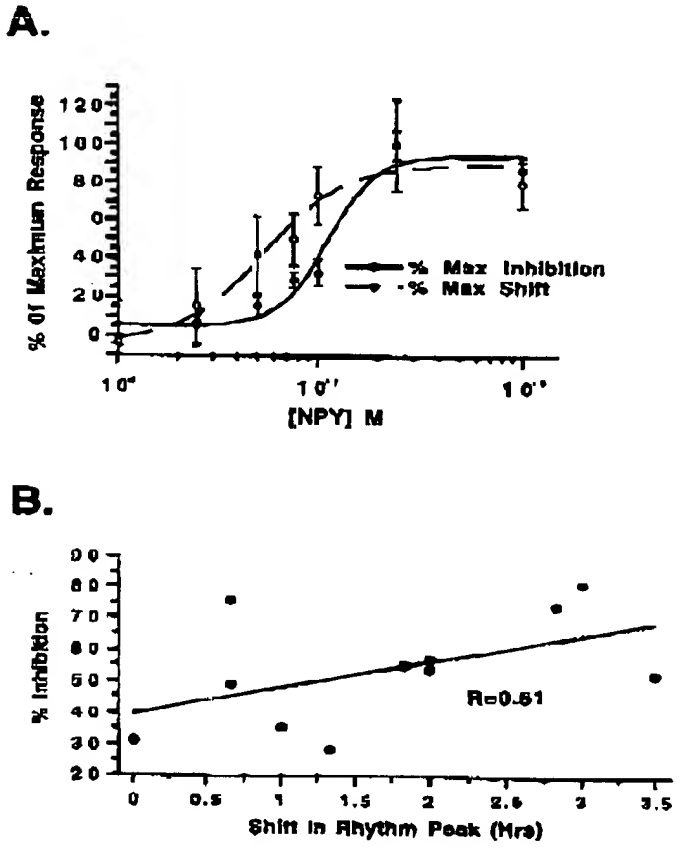

Figure 4. Physiological and pharmacological measures indicate that direct inhibition and phase shifting may be mediated by different substrates. *A*, Concentration–response relationships for phase shifting (*dashed line, open circles*) and direct inhibition (*solid line, filled circles*) produced by NPY application (10 nM–1.0 μM) at CT 7.5–8.5 on the day of slice preparation. Values are mean ± SEM. The EC_{50} values for both measures are provided in Results. The EC_{50} estimates indicate a twofold increase in sensitivity for phase shifting by NPY. The group size ranged between 6 and 22 slices for each NPY concentration. *B*, No significant linear correlation was found between the shift in the peak on day 2 and the degree of NPY-induced inhibition on day 1 resulting from application of 1.0 μM NPY.

pounds were applied on the day of slice preparation, and the peak times presented refer to the peak on day 2.

Effects of NPY application: concentration–response and lack of correlation between inhibition and phase advance

Application of NPY (1.0 μM) to slices at CT 7.5–8.5 on the day that slices were cut and placed in the recording chamber, a period of high SCN circadian sensitivity to NPY (Medanic and Gillette, 1993), resulted in a direct, significant, and reversible inhibition (>50%) of cell discharge in the SCN and a significant mean shift in the activity rhythm peak (advanced 1.74 ± 0.23 hr) measured on the second day (Figs. 2*B*, 3, 4, 5*A*). This level of phase shifting is the greatest level of alteration in peak timing produced by any putative SCN modulator, including melatonin and GABA modulators, that we have observed using this technique (our unpublished observations) and the results were very consistent.

In an initial attempt to characterize these effects, concentration–response relationships were generated for direct inhibition and phase shifting by NPY (Fig. 4*A*). Different NPY concentrations were applied to different groups of slices. A modest (twofold) difference was observed in the EC_{50} values generated for the two response measures by logistic fits of the resulting curves. The estimated EC_{50} for the phase shift produced by NPY was 54 nM, and the estimated EC_{50} for the inhibition of cell firing by NPY was 113 nM. A similar relationship was observed for maximum phase shifting by NPY; maximum phase shifts were obtained with NPY concentrations estimated to be ≥100 nM, whereas maximum inhibition was produced at approximately the same concentrations.

In a group of slices that were exposed to NPY at CT 7.5–8.5 (1.0 μM, $n = 11$), we examined the possible relationship between direct inhibition and the shift in the rhythm peak on day 2. There was no significant relationship between the level of inhibition and the phase advance observed on the next subjective day (Fig. 4*B*).

Effects of NPY application: phase–response relationships

The phase–response relationship for phase shifting by NPY has previously been determined in rat SCN *in vitro* (Medanic and Gillette, 1993; Shibata and Moore, 1993). In these previous studies application of NPY at or near CT 6–8 produced maximal advances in phase, whereas application at other time points resulted in smaller shifts. To determine the correspondence between the phase–response relationship(s) of the phase shifts and neuronal inhibition resulting from NPY administration, 1.0 μM NPY was applied at four time points (including the group at CT 7.5–8.5) to different groups of slices. Whereas a phase–response relationship was observed for phase advances produced by NPY (Fig. 5*A*), no such relationship for direct NPY-induced neuronal inhibition was observed (Fig. 5*B*). NPY applied at CT 7.5–8.5 and 9.0–10.0 produced the greatest phase advance, and NPY applied at this time point also produced robust inhibition. NPY application at two of the time points selected, CT 2.5–5.5 and CT 10.5–11.5, produced no significant phase shift (indicating a very sharp phase–response relationship), but inhibition produced by NPY was similar to that produced at other times (Fig. 5*C*,*D*).

Bicuculline application: effects on NPY phase shifts and inhibition

To determine whether the underlying mechanism of both NPY-induced phase shifts and direct inhibition involved participation of $GABA_A$ receptor-mediated events, the $GABA_A$ receptor antagonist BIC (10 μM) was applied to slices for 1 hr before the addition of vehicle or NPY (0.1 or 1.0 μM) at CT 7.5–8.5. BIC at this concentration produced an insignificant increase in firing rates (<5%) relative to untreated control slices, and the mean peak of the rhythm on day 2 was not significantly different from that of control in slices exposed only to BIC at 10 μM (peak CT, 6.39 ± 0.49; $n = 3$). BIC application at this concentration significantly antagonized (>70%) inhibition of SCN neuronal activity produced by application of the $GABA_A$ receptor agonist muscimol (our unpublished observations). The phase shift produced by both concentrations of NPY was significantly reduced by BIC (Fig. 6*A*). However, the NPY-induced depression of cell discharge in the SCN was not significantly affected (Fig. 6*B*).

Effects of NPY receptor analogs and NPY fragments

Our initial results strongly suggested that different NPY receptors mediated the phase advance and neuronal inhibition produced by application of NPY *in vitro*. To further test this hypothesis by differentiating these responses pharmacologically, a series of

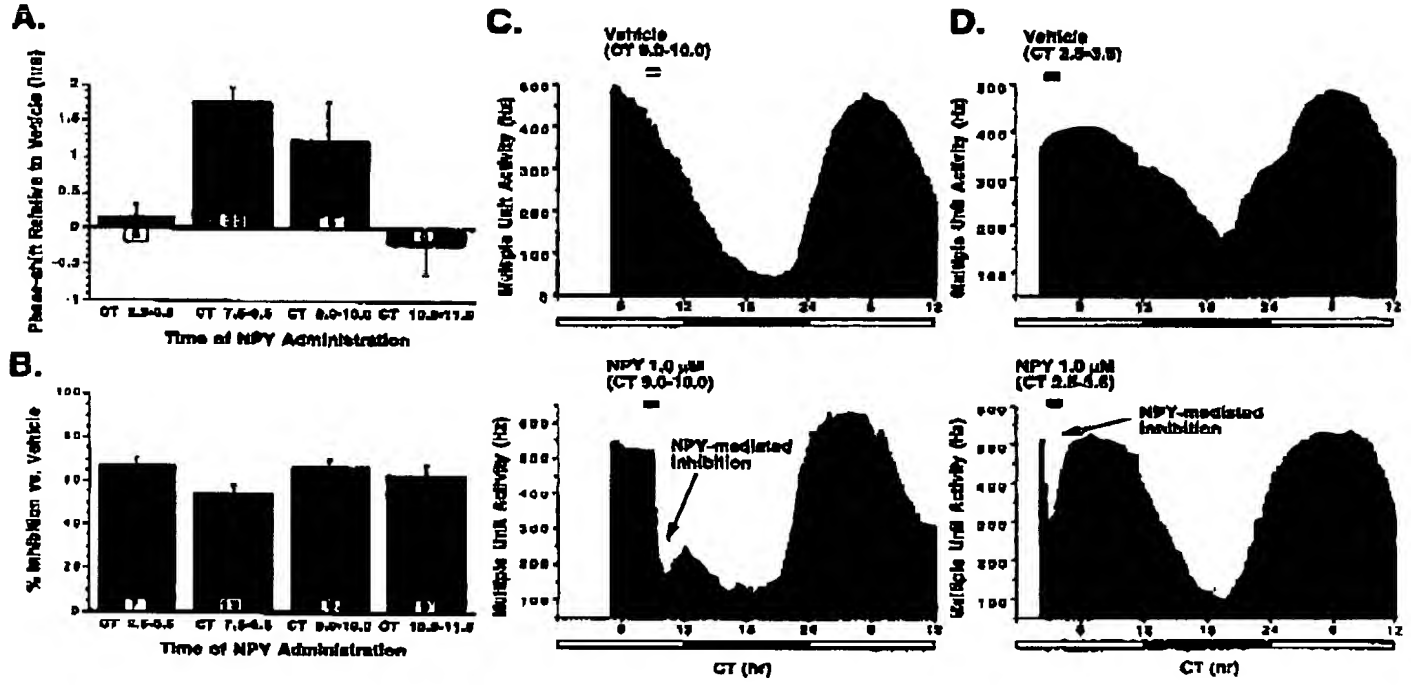

Figure 5. Effects of application time (in CT) on the response to NPY (1.0 μM). *A*, Phase–response relationship for the NPY-induced phase shift. Significant phase advances by 1.0 μM NPY were produced by application at CT 7.5–8.5 and CT 9.0–10.0 (ANOVA with Fisher's protected LSD, $p < 0.05$), with no significant shift produced by application at either CT 2.5–3.5 or CT 10.5–11.5. *B*, Lack of a phase–response relationship for direct inhibition of cell discharge by NPY in the same slices used for the phase shift experiments in *A*. Note that all of the applications were made at time points when cell discharge was within 3–5 hr of the peak, and percent inhibition values reflected similar absolute inhibition values. In this and all subsequent bar graphs the group *numbers* are indicated in the *boxes* associated with each bar. *C*, Traces depict the effects of vehicle (*top trace*) or NPY (1.0 μM, *bottom trace*) administration to slices at CT 9.0–10.0. Note the phase advance and discharge inhibition observed in the NPY-treated slice. *D*, Similar to *C*, except that vehicle (*top trace*) or NPY (1.0 μM, *bottom trace*) was applied at CT 2.5–3.5. Note that despite significant inhibition, no phase advance is observed in this slice. *Light* and *dark bars* represent the light/dark cycle before slice preparation

NPY peptide analogs and NPY peptide fragments reported to interact with different and known affinities at NPY receptors was applied to SCN slices at CT 7.5–8.5. All of these compounds were applied at 1.0 μM. These included NPY 13–36 and C2-NPY, which have high affinities for Y2 receptors; PYY, which has good affinity for both Y1 and Y2 receptors; and Leu-Pro-NPY, which has relatively higher affinity for Y1 receptors. NPY, PYY, and Leu-Pro-NPY also have high affinities for the recently reported Y5 receptor, whereas NPY 13–36 and C2-NPY have much lower affinities at this receptor. Relative affinities are taken from values published by Gerald et al. (1996).

Application of NPY 13–36, C2-NPY, and PYY produced significant phase advances in the peak of discharge rhythms, whereas Leu-Pro-NPY produced no significant shift (Fig. 7*A*). This suggests that the phase advance produced by NPY was likely attributable to interaction with a Y2 receptor, as has been indicated from previous studies, whereas interaction with a Y1 receptor and probably a Y5 receptor did not shift the rhythms. Significant levels of direct inhibition were produced by both PYY and Leu-Pro-NPY but not by NPY 13–36 and C2-NPY (Fig. 7*B*). This indicated that the direct inhibition was not produced by Y2 receptors but was probably produced by interaction with Y1 or Y5 receptors or both.

BIBP-3226: effects on NPY phase shifts and inhibition

To test for the possible involvement of Y1 receptors in direct inhibition of the SCN by NPY, the potent and specific Y1 receptor antagonist BIBP-3226 (10 μM) was coadministered with NPY (BIBP-3226 was present for 30 min before the coadministration of the antagonist and NPY) and compared with the effects of NPY application in the absence of this antagonist. BIBP-3226 had no detectable effects of its own and affected neither the degree of phase advance produced by NPY (Fig. 8*A*) nor the degree of inhibition observed in response to NPY (Fig. 8*B*). The lack of any detectable effect of this high concentration of the potent and specific Y1 antagonist suggested that Y1 receptors were unlikely mediators of the direct inhibitory effects of NPY.

Effects of Y5 receptor activation

D-Trp-NPY is a specific Y5 receptor agonist, although it is not very potent. D-Trp-NPY was applied at 1.0 and 5.0 μM to groups of slices, and its effects on the peaks of neuronal activity rhythms and discharge rates were recorded. Whereas this peptide had little effect on either measure at 1.0 μM (Fig. 9*A*,*B*), it produced significant inhibition of firing at 5.0 μM (Fig. 9*B*) with no concomitant effect on the phase of the rhythm (Fig. 9*A*). Comparison of the time course of the effects of NPY and D-Trp-NPY, relative to vehicle, demonstrate the same long time course of the resultant inhibition (Fig. 9*C*).

DISCUSSION

NPY in the SCN

Previous studies indicated that NPY had significant effects *in vitro* and *in vivo* on the timing of circadian rhythms in cell discharge

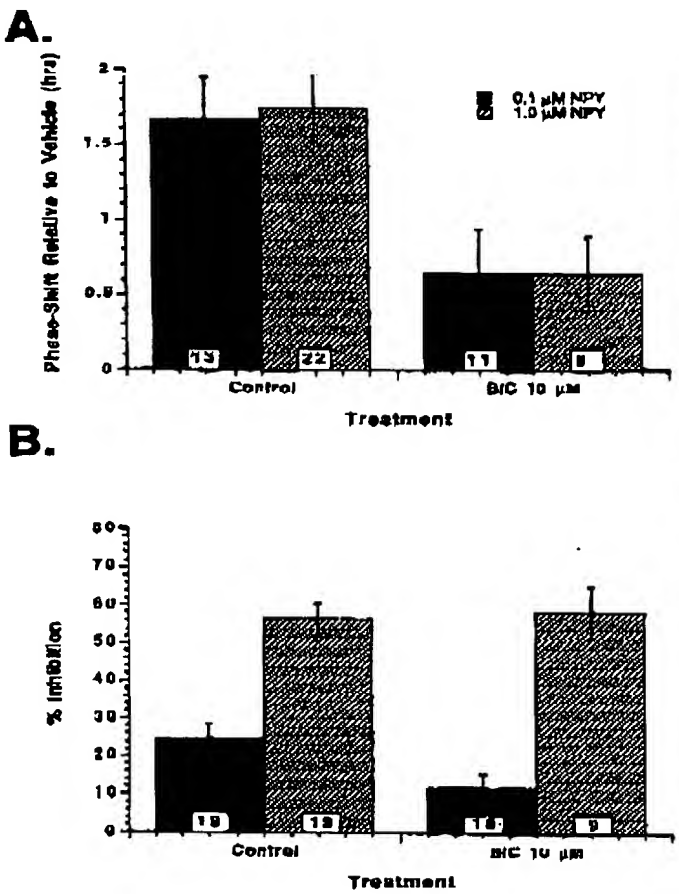

Figure 6. The effects of the $GABA_A$ receptor antagonist bicuculline (*BIC*) on NPY-induced phase shifting and direct inhibition. *A*, BIC (10 μM) significantly attenuated the phase shift produced by both 100 nM (*black bars*) and 1.0 μM (*striped bars*) NPY applied at CT 7.5–8.5 (ANOVA, $p = 0.001$; Fisher's protected LSD, $p < 0.01$). *B*, Direct inhibition was not significantly attenuated by BIC, although there was some diminution of the response to 100 nM NPY.

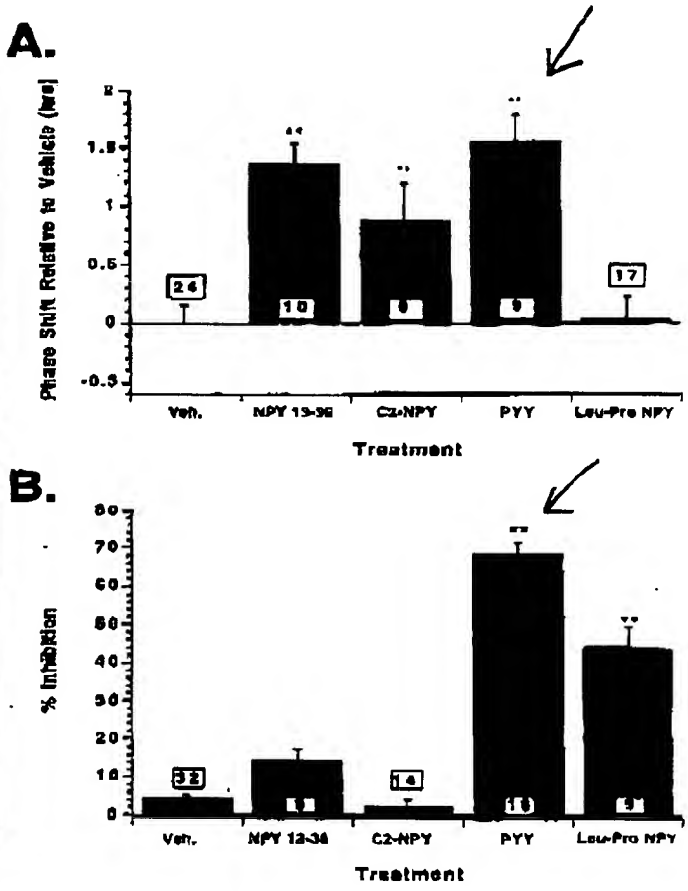

Figure 7. A series of peptidergic NPY analogs and fragments, including PYY (interacts with Y1, Y2, and Y5 NPY receptors), NPY 13–36 (a Y2-preferring agonist), [Leu^{31},Pro^{34}]-NPY (a Y1- and Y5-preferring agonist), and C2-NPY (a Y2-preferring agonist), was applied to SCN slices at CT 7.5–8.5 (all at 1.0 μM), demonstrating that although all of the peptides were active in altering at least one of the dimensions of SCN physiology, only NPY and PYY significantly affected both measures, relative to slices treated with vehicle. The Y2-preferring agonists produced a significant phase advance (*A*) but did not significantly inhibit cell firing during their application (*B*). Conversely, the Y1 agonist [Leu^{31},Pro^{34}]-NPY produced significant inhibition but no phase shift. These data suggested that Y1 or Y5 receptors or both contributed to the inhibition of firing by NPY. ANOVA with Fisher's protected LSD, $^{**}p < 0.01$.

and behavior (Medanic and Gillette, 1993; Shibata and Moore, 1993; Biello et al., 1994; Huhman and Albers, 1994). These interactions, and in particular the phase shifts produced by NPY, were probably mediated by the Y2 receptor, as indicated by studies with NPY peptidergic agonists (Golombek et al., 1996; Huhman et al., 1996). The effects of NPY on cell discharge, however, have not been well characterized. In initial studies of the direct effects of NPY administration on the firing of single neurons in SCN, NPY was reported to excite or to have no effect on the majority of cells tested, with only a minority of cells inhibited (Mason et al., 1987; Shibata and Moore, 1988; Liou and Albers, 1991). In recent studies with whole-cell recordings of the direct effects of NPY on neurons in the SCN, however, NPY was found to produce significant and prolonged inhibition of synaptic activity and calcium levels in SCN neurons in culture and in SCN slices, possibly mediated by multiple NPY receptor subtypes expressed in cell bodies and presynaptic terminals (Chen and van den Pol, 1996; van den Pol et al., 1996). NPY has other effects on SCN neurons, including the depression of GABA-mediated calcium transients in developing SCN neurons, an effect that is mimicked by Y1 and Y2 receptor agonists (Obrietan and van den Pol, 1996). The latter additional data suggested that the effects of NPY on these important mediators of circadian rhythmicity were complex, with at least two major types of action, phase shifting and direct synaptic and/or cellular inhibition. The results of the present study indicate that in the same populations of SCN neurons in which NPY produced significant phase shifts, the peptide produced significant and long-lasting inhibition of a majority of the sampled neurons.

NPY potently phase advanced the peak of the circadian rhythm of cell discharge (maximum mean phase advance, 1.74 ± 0.23 hr at 1.0 μM) and reversibly depressed cell discharge in SCN slices when applied at CT 7.5–8.5. The results of several of the exper-

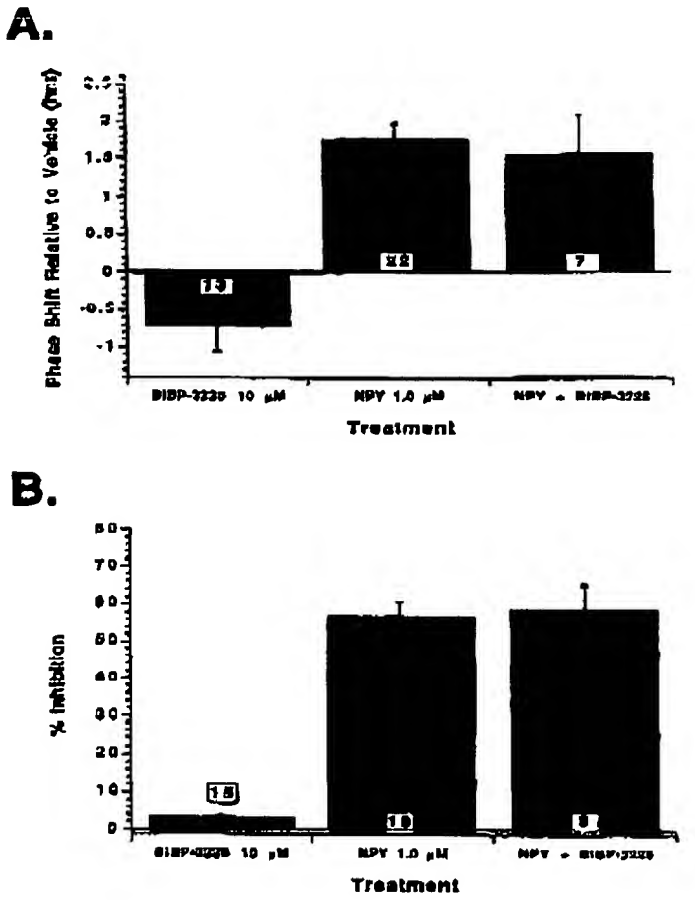

Figure 8. The specific NPY Y1 receptor antagonist BIBP-3226 (10 μM) did not significantly attenuate either the phase shift (*A*) produced by 1.0 μM NPY when they were coapplied ($n = 7$) at CT 7.5–8.5 or the direct inhibition of cell discharge (*B*) recorded in the same slices. BIBP-3226 at this high concentration ($n = 13$) did not produce a significant phase shift or depression of cell firing when applied alone. ANOVA with Fisher's protected LSD, phase shift and inhibition produced by NPY and NPY plus BIBP-3226, all $p < 0.01$.

iments in this study suggest that the observable effects of NPY application may be mediated by different receptors. NPY was applied at different times on the day of slice preparation. A significant phase advance was observed when NPY was applied at CT 7.5–8.5 and CT 9.0–10.0 but not when NPY was applied at CT 2.5–3.5 or CT 10.5–11.5 and was consistent with the NPY phase–response curve previously generated by Medanic and Gillette (1993) using a different recording technique. This narrow phase–response window was in contrast to the effects of NPY on acute depression of cell discharge, which did not demonstrate a phase–response relationship at these time points. Whereas a more detailed analysis of the phase–response relationship of direct NPY inhibition was not performed in this study, the time points were sufficiently spread out to define the phase shift relationship described previously, and no phase response was seen. We cannot address the issue of whether at unsampled time points the direct effects of NPY may be altered; earlier studies suggested that there may be a phase–response relationship for direct cellular modulation by NPY (Mason et al., 1987; Shibata and Moore, 1988; Liou and Albers, 1991).

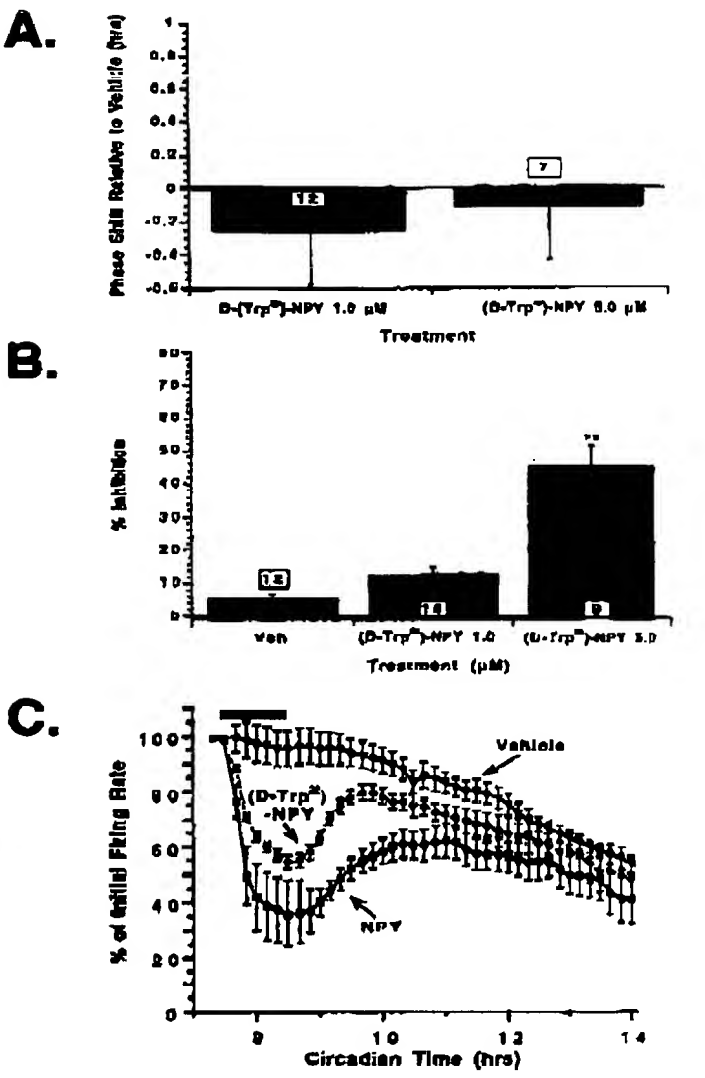

Figure 9. Unlike NPY at 1.0 μM, the specific Y5 receptor agonist [D-Trp^{32}]-NPY (1.0 and 5.0 μM, $n = 12$ and 7, respectively) did not produce a phase shift (*A*) but at the higher concentration produced significant inhibition of SCN discharge, relative to vehicle ($n = 13$) (*B*). This peptide, although specific for the Y5 receptor, is much less potent than NPY. ANOVA with Fisher's protected LSD, $^{**}p < 0.01$. *C*, Group data showing the very long time course of the inhibition of cell firing by both NPY (1.0 μM, $n = 22$) and the Y5 agonist [D-Trp^{32}]-NPY (5.0 μM, $n = 7$), relative to the vehicle control group ($n = 24$). Both peptides produced significant inhibition that recovered only very slowly; NPY was more potent, because the Y5 agonist produced only marginal inhibition at 1.0 μM and was more effective at this concentration. The maximum effect of [D-Trp^{32}]-NPY was not determined.

The $GABA_A$ receptor antagonist bicuculline significantly suppressed NPY-induced phase advances but did not significantly alter acute NPY-induced inhibition. These data again suggested that different receptors were mediating these physiological response measures. To more succinctly test this hypothesis, we

applied peptide analogs of NPY with preferences for different NPY receptors (Gerald et al., 1996), all applied at CT 7.5–8.5 at the same concentration. Only NPY, PYY (which like NPY is nondiscriminating), and Y2-preferring agonists (NPY 13–36 and C2-NPY) produced significant phase advances in the SCN circadian discharge rhythm, whereas Y1- and Y5-preferring peptides ([Leu^{31},Pro^{34}]-NPY and [D-Trp^{32}]-NPY, respectively) produced no significant phase shifts. The Y1 and Y5 agonists, however, produced significant direct cell inhibition, whereas the Y2 agonists did not produce significant inhibition. The lack of effect of the specific nonpeptidyl Y1 antagonist BIBP-3226 on NPY-mediated inhibition, coupled with the specificity of [D-Trp^{32}]-NPY for the Y5 receptor (despite its relative lack of potency) and the robust level of inhibition it produced, suggest that a major component of NPY-induced neuronal inhibition in the SCN may be mediated by Y5 receptors. An additional component could be mediated by other, currently unknown, receptors. Whereas we currently do not know the functional significance of NPY-mediated inhibition in the SCN, the involvement of a different receptor and its robust action indicate that this very likely reflects an important component of the actions of this peptide in modulating the intrinsic rhythmicity of SCN neurons, perhaps by decreasing cellular responsivity to other stimuli.

Multiunit recordings

In the present study, multiple-unit recordings were obtained from rat SCN slices to determine the effects of NPY application on circadian rhythms and acute neuronal activity rates. Whereas other studies have used multiunit recordings to follow SCN neurons through one or more circadian cycles (Bouskila and Dudek, 1993; Tcheng and Gillette, 1996; Liu et al., 1997b), this represents the first in-depth pharmacological study using this technique.

Many studies of SCN activity *in vitro* have been based on multiple episodic single-unit recordings. With this approach, a single neuron is recorded for a short interval (e.g., 1–5 min), and then the experimenter moves the extracellular electrode and chooses a different cell from which to record. Different cells then represent different time points in the course of the recording. Continuous multiple-unit recording offers two substantial advantages over single-unit recording.

First, the phase shifts observed using multiple-unit recording are similar to those in parallel experiments with drug injections *in vivo*. For example, direct injection of NPY 3–36, a Y2 receptor agonist, into the SCN area of Syrian hamsters produced a phase advance ranging from 19 to 90 min (Huhman et al., 1996). In another study of NPY injections into the SCN, where phase was partly determined by the hamster's behavioral milieu, the advances relative to saline controls were −1.5–2.3 hr (Biello et al., 1994). The phase advances reported in these *in vivo* studies were very similar to the phase shifts of 1.5–1.7 hr produced by similar compounds that we found in the present *in vitro* study with multiple-unit recording. In contrast, phase shifts reported based on the single-unit recording method were much greater, with maximal phase advances of ~4 hr (Medanic and Gillette, 1995; Golombek et al., 1996). In addition, bicuculline injections into the SCN area of rodents blocked the NPY-induced phase advances of behavioral rhythms (Huhman et al., 1995). Our similar findings in the SCN slice *in vitro*, based on data from 54 slices, are in full agreement with the previous behavioral study and lend further support to the hypothesis that NPY actions may modulate GABA activity, as previously suggested in cultures of SCN neurons (Chen and van den Pol, 1996; Obrietan and van den Pol, 1996). In contrast, a recent study using episodic single-unit recording was unable to detect this interaction of NPY with GABA (Biello et al., 1997). Thus the data recorded using the multiple-unit technique appear to more accurately predict the behavioral responses of animals after drug injections into the SCN *in vivo* than do single-unit recordings. We have observed a similar relationship with melatonin. Melatonin application *in vivo* produces only small advances, parallel to the small phase shifts (<1 hr) detected with multiple-unit recording. In contrast, reports based on single-unit recordings suggest that melatonin produces large phase shifts (Liu et al., 1997a).

A second advantage of multiple-unit recordings is that after setup, the experiment is run entirely under computer control, allowing the automated long-term recordings of a single population of SCN neurons. This eliminates any possibility of unconscious experimenter bias in the selection of cells that is a constant feature of the single-unit sampling method and that may contribute to the reports of greater magnitudes of phase shifts with the single-unit method.

Conclusion

In conclusion, using long-term multiunit electrode recording techniques, we have found that application of the neuropeptide NPY produced two distinct actions on neurons of the rat SCN *in vitro*, phase shifting and direct neuronal inhibition. Distinctions were made between these two physiological results of NPY administration in their phase–response and dose–response relationships and, more importantly, in their pharmacological profiles. Using pharmacological agents with different affinities for Y1, Y2, and Y5 NPY receptor subtypes, our results confirm that Y2 receptors mediate phase shifting by NPY, and we have determined that Y5 receptors likely contribute to direct NPY-mediated neuronal inhibition in the SCN. Other potentially important functions of NPY, such as the modulation of glutamatergic synaptic transmission from the retinohypothalamic tract, were not studied in these experiments. Finally, we have shown that long-term continuous multiunit recording of SCN electrical activity *in vitro* is a useful technique for the study of the physiology and pharmacology of these important circadian clock nuclei.

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SCIENCE DIRECT FULL-TEXT ARTICLE

Effect of peptide YY (PYY) on food-associated conflict.

Hagan MM, Moss DE.

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Peptide YY (PYY) administered centrally in rats induces powerful overeating. PYY also occurs endogenously in humans and is elevated in abstaining bulimic patients. To examine the effect of PYY in an environment that parallels some aspects of bulimia, rats were tested in a paradigm associated with approach-avoidance behavior, choosing a preferred (sweet) food paired with shock, over regular food safe from shock. PYY-treated rats chose to sustain shock to retrieve and consume the preferred food, at a significantly greater speed and quantity. The number of approaches that were met without retrieval of food due to anxiety after PYY treatment indicates that PYY increased motivation towards feeding, rather than anxiolysis. This effect of PYY in a model of conflict associated with food choice resembles aspects of bulimic binge-eating, which is characterized by the repetitive, rapid intake of food, despite anxiety associated with this behavior.

PMID: 8559783 [PubMed - indexed for MEDLINE]

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Peptide YY (PYY), a potent orexigenic agent.

Morley JE, Levine AS, Grace M, Kneip J.

Peptide YY (PYY) enhances feeding and drinking more potently than does neuropeptide Y after central administration. Chronic administration of PYY every 6 h for 48 h causes massive food ingestion. Tolerance to this effect of PYY does not appear to develop. This data suggests that PYY is one of the most potent orexigenic substances yet to be identified. PYY may play a role in the pathogenesis of bulimic syndromes.

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Neurochemistry of bulimia nervosa.

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Normal weight bulimia nervosa, a disorder of unknown etiology, is characterized by bingeing and purging behavior, disturbances of mood, and neuroendocrine abnormalities. Bulimic women have alterations of neurotransmitter systems known to contribute to the modulation of feeding, mood, and neuroendocrine function. Bulimic patients have increased cerebrospinal fluid concentrations of peptide YY (PYY), a peptide which is a potent stimulant of feeding in experimental animals. It has been suggested that increased brain PYY activity could contribute to the powerful and uncontrollable drive of bulimic patients to binge. It also has been reported that bulimics have impaired satiety and secretion of cholecystokinin, a peptide known to induce satiety and reduce food intake in animals and humans. Most data show that bulimic women have alterations of serotonin and norepinephrine activity. In animals, serotonin appears to have effects on eating behavior (inhibition) that are opposite to the actions of endogenous norepinephrine (activation) at alpha 2 receptors in the hypothalamus. Bingeing behavior is consistent with an overactivity of the hypothalamic alpha-noradrenergic system, an underactivity of hypothalamic serotonergic systems, or a combination of both defects. In summary, it is possible that bulimic patients have a trait-related disturbance of one or more neurotransmitter systems that could cause their appetitive dysregulation. Alternatively, these neurotransmitter disturbances may be secondary to extremes of dietary intake. Nonetheless, such neurotransmitter disturbances may contribute to a high recidivism rate. That is, bulimic patients could enter a vicious cycle in which pathologic feeding sustains and provokes continued pathologic feeding behavior. Moreover, the self-reinforcing effects of bulimia, such as decreased anxiety or food craving, may be mediated through behavior-induced changes in neurotransmission.

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